CLAIMS

1. Method for identifying Tr1-regulatory lymphocytes present in a biological sample comprising lymphocytes, characterized in that it comprises the following steps:

a) determining the simultaneous presence of the expression products by said lymphocytes of genes encoding the CD4 molecule and all of the molecules of group A, wherein said group A is made up of molecules CD18 and/or CD11a, and CD49b; and

b) identifying, as Tr1-regulatory lymphocytes, the lymphocytes that simultaneously express the genes encoding the CD4 molecule and all of the molecules of group A.

2. Method according to claim 1, characterized in that:

- step (a) involves the comparison of the expression by said lymphocytes of at least one gene selected from the genes encoding the molecules of following group B: CD11a, CD18, PSGL-1, PECAM-1 and alphaV/beta3, wherein said expression is compared with the expression of said same gene by Th1 or Th2 lymphocytes; and
- step (b) involves the identification, as Tr1-regulatory lymphocytes, of the lymphocytes that overexpress at least one of said genes encoding the molecules of group B.

3. Method according to claim 2, characterized in that step (a) involves the comparison of the expression of at least two of said genes of group B and in that step (b) involves the identification, as Tr1-regulatory

lymphocytes, of the lymphocytes that overexpress said two genes of group B.

4. Method according to claim 3, characterized in that step (a) involves the comparison of the expression of all of the genes of group B and in that step (b) involves the identification, as Tr1-regulatory lymphocytes, of the lymphocytes that overexpress all of the genes of group B.

5. Identification method according to any one of claims 1 to 4, characterized in that step (a) consists of determining, additionally and simultaneously, the presence of the expression product by said lymphocytes of the gene encoding the CD3 molecule and in that step (b) consists of identifying, as Tr1-regulatory lymphocytes, the lymphocytes that also simultaneously express the gene encoding the CD3 molecule.

6. Method according to any one of claims 1 to 5, characterized in that step (a) consists of determining the simultaneous presence of said molecules of group A expressed at the surface of said lymphocytes.

7. Method according to claim 6, characterized in that step (a) consists of determining the simultaneous presence of said molecules expressed at the surface of said lymphocytes by means of antibodies specific to said molecules.

8. Method according to claim 7, characterized in that said specific antibodies are marked with a marker capable of being detected directly or indirectly.

9. Method according to claim 8, characterized in that each of said antibodies is marked by a different marker.

10. Method according to claim 8 or 9, characterized in that said markers are fluorescent and are selected from the group consisting of fluorescein isothiocyanate (FITC), or allophycocyanin (APC), phycoerythrin-cyanin 5 (PC5), phycoerythrin (PE), green fluorescent fluorescein diacetate, calcein AM and red fluorescent tetramethyl rhodamine.

11. Method according to any one of claims 6 to 10, characterized in that step (a) consists of determining the simultaneous presence of said molecules of group A expressed at the surface of said lymphocytes and implemented by flow cytometry.

12. Method according to claim 10 or 11, characterized in that step (a) of said method consists of determining, for the CD18 molecule, the presence of a CD18bright fluorescence intensity.

13. Method according to any one of claims 2 to 12, characterized in that:

- in step (a), the comparison of the expression by said lymphocytes of at least one gene encoding the molecules of group B is carried out by comparing the amount of mRNA expressed for said gene; and
- step (b) involves identifying, as Tr1-regulatory lymphocytes, the lymphocytes that overexpress the mRNA of said gene.

14. Method according to claim 13, characterized in that the amount of mRNA is measured by quantitative RT-PCR.

15. Method according to any one of claims 1 to 14, characterized in that the biological sample is from a peripheral blood sample or an inflammatory organ in a subject.

16. Method according to claim 15, characterized in that the sample is taken from a subject affected or likely to be affected by an autoimmune or inflammatory disease.

17. Method according to claim 16, characterized in that said subject has Crohn's disease or multiple sclerosis.

18. Method according to any one of claims 1 to 14, characterized in that the biological sample is obtained from a method for *in vitro* preparation of Tr1-regulatory lymphocytes using a population of lymphocytes obtained from a sample of a subject.

19. Method according to claim 18, characterized in that the Tr1-regulatory lymphocyte preparation method comprises at least one step of activating CD4+ T lymphocytes of said lymphocyte population in the presence of an antigen and interleukin 10.

20. Method according to claim 18, characterized in that the Tr1-regulatory lymphocyte preparation method comprises the following steps:

(a) obtaining a biological sample containing artificial antigen-presenting cells that express a molecule of the HLA class-II system and a human LFA-3 molecule and that do not express any co-stimulation molecules B7-1, B7-2, B7-H1, CD40, CD23 or ICAM-1;

(b) activating, *in vitro*, the CD4+ T lymphocytes of said lymphocyte population in the presence of the selected antigen, presented by artificial antigen-presenting cells obtained in (a); and

(c) collecting, from said lymphocytes, an activated CD4+ lymphocyte population comprising at least

10 % Tr1 lymphocytes specific to the selected antigen.

21. Method according to claim 18, characterized in that the Tr1-regulatory lymphocyte preparation method comprises the following steps:

(a) obtaining, *in vitro*, a population of human progenitor cells capable of differentiating into dendritic cells;

(b) placing said human progenitor cells in a culture in the presence of IL-10 so as to obtain a population of said dendritic cells; and

(c) placing said human lymphocyte population in the presence of the dendritic cell population obtained in (b).

22. Method according to any one of claims 1 to 5, characterized in that the expression products by said lymphocytes of genes encoding the molecules of group A are mRNAs, and in that, in step (a), the determination of the simultaneous presence of said mRNA is performed by RT-PCR.

23. Method for quantification of Tr1-regulatory lymphocytes present in a biological sample comprising lymphocytes, characterized in that it comprises the steps of:

(a) identifying Tr1-regulatory lymphocytes using an identification method according to anyone of claims 1 to 21; and

(b) determining the proportion of Tr1-regulatory lymphocytes identified in (a) with respect to the total amount of lymphocytes or a particular fraction of lymphocytes, present in said biological sample.

24. Method for *in vitro* prognosis or diagnosis of an autoimmune or inflammatory disease in a subject, using a biological sample previously taken from said subject, characterized in that it comprises the following steps:

(a) determining the proportion of Tr1-regulatory-lymphocytes present in said biological sample with respect to the total amount of lymphocytes or a particular fraction of lymphocytes, according to the quantification method of claim 23; and

(b) comparing the proportion of said Tr1-regulatory lymphocytes obtained in step (a) with that present in a biological sample taken from a healthy subject.

25. Method for *in vitro* prognosis or diagnosis of an autoimmune or inflammatory disease according to claim 24, characterized in that, in step (b), a reduction in said proportion is observed in the subject to be tested.

26. Method for enrichment of Tr1-regulatory lymphocytes present in a biological sample comprising lymphocytes, characterized in that it comprises the following steps:

(a) identifying the Tr1-regulatory lymphocytes using the identification method according to any one of claims 1 to 22; and

(b) removing a significant portion of the lymphocytes not simultaneously having said molecules from said sample.

27. Use of a population of Tr1-regulatory lymphocytes enriched by an enrichment method according to claim 26 for the production of a drug intended to prevent and/or treat an autoimmune or inflammatory disease.

28. Use according to claim 27, characterized in that the Tr1-regulatory lymphocytes are administered at the level of an inflammation area.

29. Use according to claim 27 or 28, characterized in that the Tr1-regulatory lymphocytes are administered with an antigen capable of activating said lymphocytes *in vivo*.

30. Use according to claim 27 or 28, characterized in that the Tr1-regulatory lymphocytes have previously been activated *in vitro* or *in vivo*.

LEGENDS

Figure 1

Production de cytokine	Cytokine production
Incorporation de Thymidine	Thymidine Incorporation
Aucun	None
Epaississement de l'oreille	Thickening of the ear
Heures	Hours
Cellules T CD4	CD4 T Cells
% de cellules totales	% of total cells

Figure 2

Production de cytokine	Cytokine production
Maladie de Crohn	Crohn's disease
% de cellules T CD4+	% of CD4+ T cells
Sain	Healthy

Figure 3

Colon	Colon
MLN	MLN

Figure 4

Cellules fluorescentes	Fluorescent cells
10^6 cellules	10^6 cells
Infiltrat total	Total infiltrate
CD4+ infiltrantes	infiltrating CD4+
Côlon inflammé	Inflamed colon
Rate	Spleen
Ganglions lymphatiques mésenteriques drainants	Draining mesenteric lymph nodes

Figure 5

Epaississement de l'oreille	Thickening of the ear
Jours	Days
Oreille traitée avec le véhicule	Ear treated with the carrier
Oreille traitée avec oxazolone	Ear treated with oxazolone
Oreilles Inflammées	Inflamed ears
Ganglions lymphatiques drainants	Draining lymph nodes
Rate	Spleen
Ganglions lymphatiques périphériques drainants	Draining peripheral lymph nodes
Nombre total de cellules fluorescentes récupérées/10^6	Total number of fluorescent cells collected/10^6

Figure 6

Cellules adhérentes	Adherent cells
Champ	Field

Figure 7

Sélectine P	P-Selectin
Anti-chaîne	Anti-chain
Evénements de roulement	Rolling events
Cellules adhérentes	Adherent cells
Champ	Field
% d'inhibition	% inhibition
Cellules fluorescentes	Fluorescent cells
Epaississement de l'oreille	Thickening of the ear

Figure 8

Taux d'augmentation croissante	Rate of increase
Clones	Clones
Populations	Populations

Figure 11

Nombre de fois d'augmentation par rapport à la négative	Number of times of the increase with respect to the negative

Figure 12

Cellules T différentiées avec	T cells differentiated with
Rien	Nothing
Cellules T différentiées	Differentiated T cells

Figure 13

Milieu	Medium
CMH classe I	Class I MHC

Figure 14

Aucune	None
Cellules différentiées	Differentiated cells

Figure 15

Lymphocytes	Lymphocytes
Monocytes	Monocytes